Perbandingan Pemeriksaan Histopatologi antara Efek Pemberian Mannitol Injeksi dengan Asam Askorbat Injeksi terhadap Derajat Kelangsungan Hidup Sel pada Cedera Reperfusi Flap Tikus

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ABSTRAK

Pendahuluan. Cedera reperfusi memiliki dampak praktis pada bidang orthopaedi dan traumatologi dan operasi rekonstruktif dengan tujuan penyelamatan ekstremitas. Salah satu penyebab cedera reperfusi adalah adanya peningkatan radikal bebas. Mannitol dan asam askorbat adalah antioksidan yang dapat digunakan untuk membatasi terjadinya cedera reperfusi dengan cara mengikat radikal bebas. Untuk itu perlu dilakukan penelitian membandingkan efek pemberian antara injeksi mannitol dengan injeksi asam askorbat terhadap cedera reperfusi pada tikus untuk mengetahui manakah yang lebih baik untuk menjadi standard pengurangan efek cedera reperfusi.

Bahan dan cara kerja. Desain penelitian ini adalah eksperimental dengan rancang acak lengkap. Sampel berupa tikus jenis Wistar jantan sejumlah 27 ekor yang dibagi menjadi 3 kelompok sama rata yang setiap kelompok mendapat perlakuan injeksi NaCl 0,9% intravena sebagai kontrol, injeksi mannitol intravena dengan dosis 200 mg/kgBB, dan injeksi asam askorbat intravena dengan dosis 350 mg/kgBB. Data berupa rerata jumlah sisa inti sel yang hidup pada setiap lapang pandang dalam pengamatan histopatologis. Analisis dilakukan dengan uji ANOVA dilanjutkan dengan uji Bonferroni.

Hasil. Hasil penelitian menunjukkan bahwa terdapat perbedaan yang bermakna ($p<0,05$) baik pada kelompok injeksi mannitol (12.22±7.23) dibandingkan kelompok injeksi NaCl (4.44±2.30), pada kelompok injeksi asam asorbat (19.22±1.92) dibandingkan kelompok injeksi NaCl, serta pada kelompok injeksi asam askorbat dibandingkan kelompok injeksi mannitol.

Kesimpulan. Dari hasil penelitian dapat disimpulkan bahwa, penggunaan injeksi asam askorbat intra vena mempunyai efektifitas yang lebih baik dibandingkan injeksi mannitol intra vena dalam membatasi terjadinya nekrosis pada cedera reperfusi pada *flap* tikus sehingga meningkatkan keberhasilan *flap*.

Kata kunci: reperfusi, inti sel otot lurik hidup, mannitol, asam askorbat

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Comparison of Histopathologic Examination between Mannitol Injection and Ascorbic Acid Injection Effect in Cell Survival Degree of Reperfusion Injury at Rat's Flap

ABSTRACT

Introduction. Reperfusion injury has a practical impact on the field of orthopedics and Traumatology and reconstructive surgery with the aim of saving the limb. One cause of reperfusion injury is the presence of increasing free radicals. Mannitol and ascorbic acid are antioxidants that can be used to limit the occurrence of reperfusion injury by binding the free radicals. This study was performed to compare the effects of administration of mannitol injection with injection of ascorbic acid on reperfusion injury in rats to find out which is better to be a standard reduction of the effects of reperfusion injury.

Materials and methods.This study design was randomized controlled trial using 27 samples of male wistar rats who were divided equally into 3 groups of each group received treatment of NaCl 0.9% intravenous injection as a control, mannitol intravenous injection 200 mg/kgBW and intravenous injection of ascorbic acid 350 mg/kgBW. The data is the average number of remaining cell core living in visual field in histopathological observations. The analysis was performed by ANOVA followed by Bonferroni test.

Results. The results showed that there was significant difference ($p<0.05$) at both injection mannitol group (12.22±7.23) compared with NaCl injection group (4.44±2.30), on asorbat acid injection group (19.22±1.92) compared with NaCl injection group, and ascorbic acid injection group than the injection group mannitol.

Conclusions. From the results it can be concluded that the use of intravenous injections of ascorbic acid has a better effectiveness compared to intravenous injection of mannitol in limiting the occurrence of necrosis on reperfusion injury in rat flap thereby increasing the success of the flap.

Key words: reperfusion, skeletal muscle cell core living, mannitol, ascorbic acid

Introduction

Reperfusion injury have a practical impact on the field of Orthopaedi and traumatology and reconstructive surgery with the goal of limb salvage vascularization. Reduction of reperfusion injury is expected to help improve wound healing, soft tissue viability or flap, muscle contractility, and bone healing.[1]

According to Zaccaria, et al.,[4] reperfusion injury is caused by free radicals. Free radicals are a chemical substance that has one or more unpaired electrons in its outer part. This molecule is very unstable and tend to hold a chain reaction that resulted in injury to the tissue.[2-4]

Mannitol or hexan-1 ,2,3,4,5,6-hexol ($C_6H_{14}O_6$) is an osmotic diuretic agent and a weak renal vasodilator. Mannitol is also an antioxidant that acts to take radical hidroxyl that mannitol can be used to reduce cell death due to reperfusion injury.[2,4,5] When injected through a vein, mannitol works limited to the outside of cells (extra cellular).

Oredsson[5] in 1994 examined the effect of mannitol on the reperfusion injury and concluded mannitol may reduce necrosis of muscle due to the binding effect of free radicals from mannitol. Schanaider[4] also investigated the usefulness of mannitol on reperfusion injury to the skin island rectus abdominis muscle flap in rats and concluded that mannitol serves as a binder of free radicals can protect the flap on the research conducted up to 75% of injuries reperfusion.

Vitamin C or ascorbic acid ($C_6H_8O_6$) is a vitamin that has many functions. One is as an antioxidant that can scavenge free radicals, so that ascorbic acid can also be used to reduce cell death due to reperfusion injury.[3] Ascorbic acid can work inside and outside the cell in the fight against free radical damage.

Zaccaria[3] in 1994 examined the effect of vitamin C against reperfusion injury in epigastric flap model in rats and concludes that vitamin C can reduce reperfusion injury after six hours of experience ischemia.

Then in 2001 and 2004, Kearns[6,7] evaluated the

usefulness of oral vitamin C on rabbit skeletal muscle and concluded that administration of vitamin C can reducing the effects of reperfusion injury.

Based on theory and previous research, we wanted to compare the effectiveness between mannitol and ascorbic acid in preventing the occurrence of reperfusion injury based on histopathology finding. In our hospital, there was also no standard reperfusion injury prevention. Hence, we wanted to compare the effect of mannitol injection with an injection of ascorbic acid on reperfusion injury in mice to determine which is better to be a standard reduction of the effects of reperfusion injury. To facilitate observation, we used a model of rectus abdominis muscle flap that has vascularization from the epigastrica inferior artery.

Materials and methods

This study is an experimental research laboratory with the design of randomized design in 27 male Wistar rats. Inclusion criteria for this study were healthy male Wistar rats aged 2 months with body weight ±300-400 g. Exclusion criteria were ulcer sores on the area studied and sick animals.

The procedures began by making a flap with a size of 3x6 cm^2 in the right abdomen with a midline as the medial border of the pedicle and blood supply in the right inferior (inferior epigastic arteries and veins). After the dissection flap, clamp was mounted on the proximal vascular pedicle (inferior epigastric artery) for three hours. The flap was then covered with moist gauze. In group I as control, normal saline fluid was injected in the left femoral vein, while group II and III received injection of the liquid mannitol 20% (3m1/kgBW) and ascorbic acid (450 mg/kgBW) respectively. All injections were performed 15 minutes prior to clamp release. In the assessment phase, animals were sacrificed after seven days, and the rectus abdominis flap was then released, taken away with part of

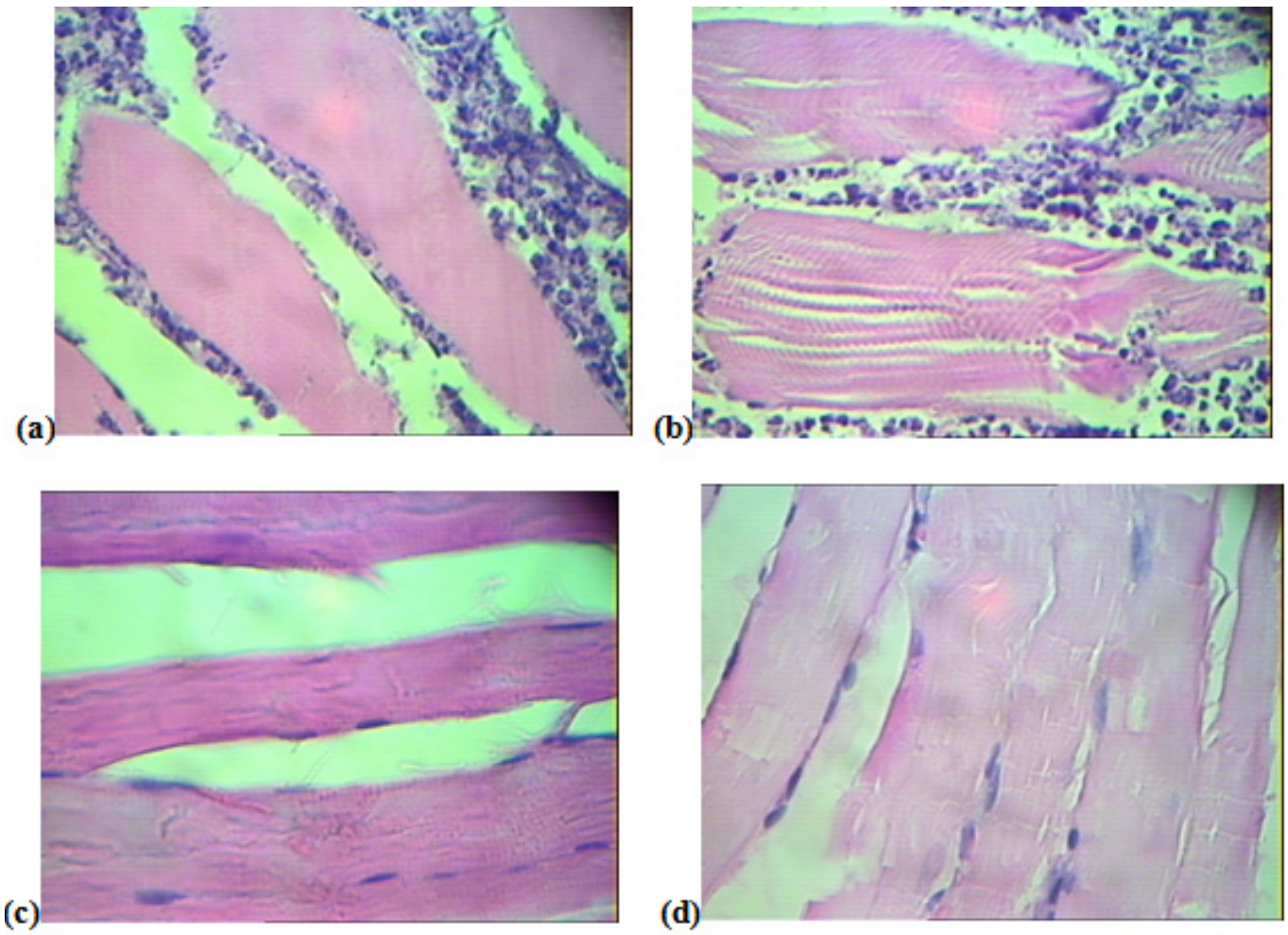

Figure 1. Skeletal muscle is examined under a light microscope by staining hematoxyline-eosine, (a) group I, (b) group II, (c and d) group III

Table 1. Comparison of living muscle cell nuclei number between the treatment groups based ion analysis of varinats

Number of living nuclei	Treatment			*p*
	Control (NaCl)	Mannitol	Ascorbic acid	
Mean (SD)	4.44 (2.30)	12.22 (7.23)	19.22 (1.92)	<0.001
Range	2.68– 6.21	6.67– 17.78	17.74– 20.70	

the vascularization, and fixed with 10% formalin solution.

The specimens were taken to the Pathology Anatomy Department of Hasan Sadikin Hospital for histopathological examination. Histological examination was performed by hematoxyline-eosine staining and examined under a light microscope by an expert pathologist (blinded) to assess the number of nuclei that are still alive (magnification x400). Data collected from the point above was performed Shapiro-Wilk statistical analysis, ANOVA test and Bonferroni multiple comparison test.

Results

The results showed different results for each test on all three kinds of treatment. Figure 1 showed examination of muscle under light microscope. Shapiro-Wilk test indicates that the data are normally distributed for all types of treatment so that parametric test was performed using ANOVA followed by Bonferroni test to determine the degree of significance between treatment groups. Statistical results of ANOVA and Bonferroni test were shown in table 1 and 2.

Comparison of average number of living nuclei between the treatment groups can be seen in figure 2. The figure shows that the group treated with intravenous mannitol injection had an average number of nuclei that live beyond the control group and the group treated with intra-venous injection of ascorbic acid has an average number of nuclei that live beyond the control group and the group treated with intra-venous injection of mannitol.

Discussions

This study assessed the process of limiting reperfusion injury by using histopathologic evaluation. Histopathological examination is one method that is considered quite easy and simple in assessing the process of limiting reperfusion injury, is also a relatively inexpensive method compared with other methods. Histopathological examination assesses reperfusion injury by seeing a evaluating the living cell nucleus.[8]

Mannitol was chosen because it can take free radicals such as hydroxyl radicals that mannitol can be used to reduce the risk of cell death caused by reperfusion injury.[2,4] Ascorbic acid was chosen because it can neutralize free radicals, so that ascorbic acid can be used to reduce the risk of cell death caused by reperfusion injury. Ascorbic acid can work inside and outside of the cells against free radical damage.[3]

The results of this study indicated that intra-venous injection of mannitol and intravenous injection of ascorbic acid had an effect in maintaining cell survival due to reperfusion injury in rat flap assessed histopathologically. Based on the number of the living cell nucleus, visible results that appear in the control group of mice was 4.44, 12.22, and 19.22 in control, mannitol and ascorbic acid group respectively. In a preliminary study, we found the average number of striated muscle cell nuclei of living by 21.

Statistically, the number of nuclei found alive in the control group and the intra-venous injection of mannitol obtained p values of <0.05, meaning that there is significant difference between the two groups in the process of limiting the occurrence of reperfusion injury. The results are consistent with previous studies which stated that the administration of mannitol may reduce necrosis of the muscle in reperfusion injury.[4,5]

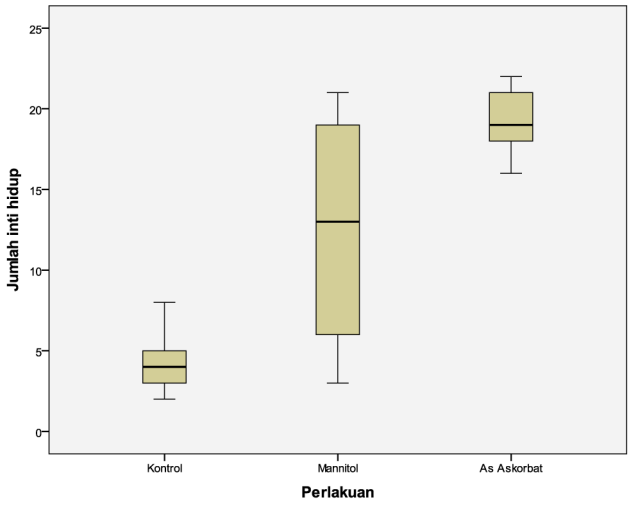

Figure 2. Comparison of the average of living muscle nuclei cells between the treatment groups

Table 2. The predictive value (p) between treatments groups based on the bonferroni test

Comparison	***p***
Control (NaCl) versus Mannitol	0.004
Control (NaCl) versus Ascorbic acid	<0.001
Mannitol versus Ascorbic acid	0.009

In this study, intra-venous injection of mannitol maintain cell viability better than the control group. The number of nuclei found alive in the control group ascorbic acid group also differs statistically ($p<0.05$), meaning that there is significant difference between the two groups in the process of limiting the occurrence of reperfusion injury. These results are also in accordance to previous studies which stated that the administration of ascorbic acid can reduce the effects of reperfusion injury.[3]

In this study the injection of intravenous ascorbic acid is shown to maintain cell viability better compared to the control group. The living cells between mannitol and ascorbic acid also differ statistically ($p<0.05$), meaning that there is significant difference between the two groups in the process of limiting the occurrence of reperfusion injury.

It endorses the hypothesis in general, in accordance with the theory put forward earlier that, both mannitol and ascorbic acid may help maintain cell viability in reperfusion injury via free radical binding process. It is said that mannitol maintains cell viability in reperfusion injury by binding to free radicals outside the cell, while ascorbic acid plays role in maintaining cell viability in reperfusion injury by binding to free radicals both inside and outside the cells.

Ascorbic acid can work both outside and inside the cell. Therefore, it is understandable that ascorbic acid has better results in limiting the occurrence of reperfusion injury.[1]

We found it difficult in terms of wound dressings that are used to cover the surgical wound. Dressing used on some subjects were often detached or damaged in the middle of the study, so that the bandage should be changed frequently. This condition occurred because the subject was too active.

Limitation to our study is that it was conducted on animal. Therefore, similar research in human subjects is still required. Another limitation to our study is due to the lack of variability in the dosage and optimal mode of administration.

Conclusions

We concluded that the use of intra-venous injection of ascorbic acid has a better efficacy compared to intravenous injection of mannitol in maintaining cell viability in reperfusion injury in rat flap.

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